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BASIC STUDIES RELATED TO THE DEVELOPMENT OF A  
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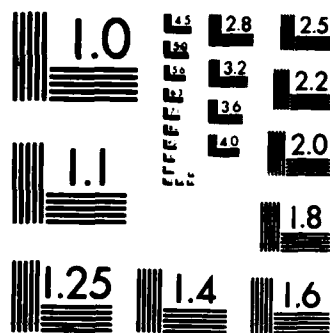
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BASIC STUDIES RELATED TO THE DEVELOPMENT OF A  
POLYVALENT MENINGOCOCCAL VACCINE

Final Comprehensive Report

J. McLeod Griffiss, M.D. and Edward H. Kass, M.D., Ph.D.

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FIELD	GROUP	SUB-GROUP	meningococcal vaccines, <u>Neisseria meningitidis</u> , capsular polysaccharides		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) <p>Two aspects of the development of a polyvalent meningococcal vaccine were studied. The first objective was to determine whether <u>Neisseria meningitidis</u> strain 8021 that produces a capsular polysaccharide with the serologic specificities of both the Y and W capsules produces its mosaic capsule with sufficient stability to make it a candidate for inclusion in vaccine. The second aim was to determine the nature of the human immune response to the group 29E (E) capsular polysaccharide. Expression of the two polysaccharide specificities by strain 8021 was not stable, and thus this polysaccharide is not a suitable vaccine candidate. Development of monovalent group Y and W vaccines has been completed. Humans were found to respond to two epitopes contained within the E polysaccharide. Response varied by individual and in Ig isotype. It was concluded that neither the E nor Z polysaccharides should be considered for inclusion in a polyvalent vaccine until their immunochemistry was.</p>					
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more completely understood. The cross-reaction observed between the E polysaccharide and enteric LPS is one of set of cross-reactions, the precise molecular basis of which remains obscure. It involves the glucosamine moiety in the LPS lipid A backbone and the galactosamine moiety of the E polysaccharide, rather than the carboxylic acid residues of dOClA substituents common to both molecules.

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## SUMMARY

This report summarizes fifteen months of a contract to study two aspects of the development of a polyvalent meningococcal vaccine. The first aspect involved Neisseria meningitidis strain 8021 that produces a capsular polysaccharide with the serologic specificities of both the group Y and group W135 capsular polysaccharides. The objective of this contract was to determine, unambiguously, whether strain 8021 produces an immunochemical mosaic molecule in addition to individual molecules with group Y and group W135 specificities. If a mosaic molecule was produced, the ratio of its hexose constituents was to be determined as well as the ratio of the two individual molecular species and any lot-to-lot variations in these ratios.

In pursuit of these objectives, three separate lots of 8021 capsular polysaccharide were prepared. Analyses of these lots revealed that the glucose content had diminished from the 15% initially reported to 3-5%, and that group Y antigenicity was correspondingly reduced. Two lots were tested as vaccines in man and found to have minimal group Y immunogenicity. We concluded that glucose content, and hence, group Y specificity, was too inconstant in 8021 capsule for it to be a useful vaccine candidate, and further studies were not carried out. During these 15 months, publications reporting the immunogenicity and optimal dose in man of group Y and group W135 vaccines were prepared. Group Y and W135 vaccines do not need further development.

The second aspect was the nature of the human response to the group 29E capsular polysaccharide. In particular, it was planned to determine if the loss in some individuals of serum bactericidal activity following vaccination resulted from preferential induction of IgA, and to confirm and extend preliminary observations that human group 29E polysaccharide antibody binds to the LPS of Enterobacteriaceae and Neisseriaceae.

The group 29E polysaccharide was shown to be both cross-reactive and cross-immunogenic with group Z polysaccharide. The molecular basis of the cross-reaction remains unclear. Vaccination with group 29E polysaccharide induced antibody to both group 29E and group Z polysaccharides. Bactericidal antibody was directed at an unique epitope of group 29E polysaccharide; bactericidal blocking antibody was directed at an epitope shared by the two polysaccharides. An isotype-specific radioimmunoprecipitin modification of the radioactive antigen binding assay was developed. Finally, the cross-reaction between group 29E polysaccharide and the core polysaccharide of the LPS of Enterobacteriaceae and Neisseriaceae was found to involve the hexosamines of the LPS lipid A backbone (glucosamine), and of the group 29E polysaccharide (galactosamine).

# FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

## I. Introduction.

This report covers 15 months of a proposed 24 month project to investigate two discrete questions that arose during prosecution of the Walter Reed Army Institute of Research program in meningococcal disease prevention using capsular polysaccharide vaccines. The two questions are reported separately.

## II. Progress Report.

### A. 8021 capsular polysaccharide:

Strain 8021 Neisseria meningitidis produces a capsular polysaccharide with the serologic specificities of both the group Y and group W135 capsular polysaccharides<sup>1</sup>. On the basis of double diffusion in gel analysis, it was concluded that this strain elaborates both individual molecules of group W135 and group Y immunochemical specificity and a molecule containing both immunochemical determinants. The mosaic data, however, were not unambiguous. It was proposed, therefore, to confirm this interpretation of the gel pattern using sequential affinity immuno-sorbent chromatography. Using this method, a mosaic molecule could be separated from molecules of individual specificity by its ability to bind to purified antibody against both of the individual determinants which had been immobilized by covalent coupling to cyanogen bromide-activated Sepharose. Antiserum raised in rabbits to prototype strains of each serogroup would be rendered monospecific by absorption with the polysaccharide of the opposite serogroup. In addition to the unambiguous demonstration of a mosaic molecule, if any, lot-to-lot variations in the ratio of molecules of individual specificities as well as mosaic molecules were to be determined. This was to be accomplished by the measurement of the ratio of glucose to galactose within molecular species, since both capsular polysaccharides are co-polymers of sialic acid and hexose, either glucose (Y) or galactose (W135).

Three separate lots of strain 8021 capsular polysaccharide were prepared, and antisera to group Y and group W135 prototype strains were raised in rabbits.

Analysis of the three lots by gas-liquid chromatography revealed that the glucose content was considerably diminished as compared to that of the prototype lots. The original lots contained ca 15% glucose; the three new lots contained circa 3-5% glucose. When analyzed by double diffusion in gel, none of the three lots reacted strongly with group Y antisera. Subsequently, two additional lots of 8021 capsular polysaccharide were prepared as vaccines at the Walter Reed Army Institute of Research and tested in adults. Neither lot was a satisfactory immunogen; induction of group Y polysaccharide antibody was no greater than that by a monovalent group W135 vaccine. It was concluded that elaboration of the group Y

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<sup>1</sup> Brandt, B.L., Pier, G.B., Goroff, D.K., Altieri, P.L., Griffiss, J. McL. Elaboration of both the group W135 and group Y capsular polysaccharides Neisseria meningitidis. J. Gen. Microbiol. 118: 39-43, 1980.



component of 8021 polysaccharide (sialic acid-glucose) was unstable and that this capsular polysaccharide was not a potentially useful vaccine candidate. Therefore, further studies were not carried out.

During these 15 months, reports of the immunogenicity and dose-response in man of group Y and W135 vaccines were prepared.<sup>2,3</sup> These polysaccharides were found to be immunogenic at doses of 5 ug. Since a divalent preparation containing 10 micrograms of total polysaccharide was fully immunogenic and without reactogenicity, further development of group Y and W135 vaccines does not appear necessary.

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- <sup>2</sup> Griffiss, J.McL., Brandt, B.L., Altieri, P.L., Pier, G.B., Berman, S.L. Safety and immunogenicity of group Y and group W135 meningococcal capsular polysaccharide vaccines in adults. *Infect. Immun.* 34:725-32, 1981.
  - <sup>3</sup> Griffiss, J. McL., Brandt, B.L., Broude, D.D. Human immune response to various doses of group Y and W135 meningococcal polysaccharide vaccines. *Infect. Immun.* 37:205-8, 1982.

## B. Immune response to 29E polysaccharide:

The human immune response to the group 29E capsular polysaccharide is distinctly and unexpectedly different from that to any other bacterial polysaccharide previously studied. Although it is immunogenic, as measured by radioactive antigen binding assay (RABA), the induced antibody is inconstantly bactericidal. More surprisingly, some individuals following vaccination experience a reduction in bactericidal titre or remain without serum bactericidal activity despite a brisk binding antibody response. Since the bactericidal capacity of induced antibody is the functional correlate of its protective capacity, the failure of this polysaccharide to induce bactericidal antibody deserves further attention. Preliminary experiments suggested that this failure might result from the preferential induction of serum IgA that blocks the bactericidal activity of serum IgG and IgM.

A second unusual aspect of the group 29E polysaccharide is the presence within it of 2-keto, 3-deoxy, octulosonic acid (KDO). This unusual carbohydrate molecule is also a part of the K13 Escherichia coli capsular polysaccharide and, most importantly, it is the linking molecule between the lipid and polysaccharide moieties of the LPS of all aerobic Gram-negative bacteria. Preliminary data suggested that a cross-reaction existed between the KDO within the group 29E polysaccharide and that within the lipopolysaccharide of Salmonella minnesota. If this proved true, the 29E polysaccharide might be capable of inducing antibody directed at the lipopolysaccharide of all aerobic Gram-negative bacteria.

The hypotheses to be tested under this contract were: 1) that group 29E polysaccharide preferentially induces serum IgA in some individuals and both IgA and lytic antibody (IgM and/or IgG) in others; 2) the induced IgA, because of its decreased serum half-life (1/5 that of IgG) declines by six weeks, unblocking induced lytic activity; 3) that whole serum bactericidal activity varies with the ratio of induced IgA to lytic antibody; and 4) that the KDO within the group 29E capsular polysaccharide immunologically cross-reacts with that within the lipopolysaccharide of the Enterobacteriaceae.

The immunologic relationship between group 29E and group Z polysaccharide was investigated using bacterial agglutination, precipitin-in-gel, RABA and immune lysis assays<sup>4</sup>. The two polysaccharides were both cross-immunogenic and cross-reactive. Demonstration of the relationship using group Z antisera depended on which assay was used. It was most readily apparent in assays of immune lysis, less apparent when precipitins were sought in gel and inapparent when bacterial agglutination, the standard assay for determining serogroup, was employed. The cross-reacting epitope was expressed 10 fold more within the group 29E polysaccharide than within the group Z polysaccharide. We conclude that inclusion of either polysaccharide in a polyvalent vaccine would obviate the need to include the other.

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<sup>4</sup> Griffiss, J.McL., Brandt, B.L. Immunological relationship between the capsular polysaccharides of Neisseria meningitidis serogroups Z and 29E. J. Gen. Microbiol. 129: 447-452, 1983.

The chemical basis of the cross-reaction was explored using inhibition of the RABA. Group 29E polysaccharide (KDO-galactosamine), *E. coli* K 13 polysaccharide (KDO-ribose), group Z polysaccharide (galactosamine-glycerol phosphate) and meningococcal group X capsular polysaccharide (glucosamine phosphate) were used as inhibitors. Group 29E and Z polysaccharides were subjected to periodate oxidation and borohydride reduction and to carbodiimide reduction. Both polysaccharides were also O deacetylated. It was deduced that the 29E polysaccharide contains two separable epitopes: one that is unique to the 29E polysaccharide and is dependent upon the carboxylic acid of the KDO residue, and one that is cross-reactive with group Z polysaccharide and dependent upon the presence of hexosamine. Antibody to the cross-reactive epitope does not distinguish between glucosamine and galactosamine.

A group 29E polysaccharide vaccine in a dose of 50 ug was injected subcutaneously into 10 adult human volunteers<sup>5</sup>. One vaccinee experienced a mild systemic reaction; one complained of moderate to severe local pain and tenderness. The vaccine induced significant homologous binding and bactericidal antibody by 2 weeks and significant binding antibody against the heterologous group Z polysaccharide by 4 weeks. Although binding antibody rose during the first 4 weeks and then declined slowly over the subsequent 4 months, bactericidal antibody response declined substantially by 4 and 8 weeks for both polysaccharides. The increase in group 29E bactericidal activity was no longer significant at 4 and 8 weeks; loss of bactericidal activity against group Z was significant by 8 weeks. Bactericidal activity again rose between 8 and 26 weeks, becoming significantly increased over prevaccination levels for group 29E and increased, though not significantly ( $P=0.085$ ), over prevaccination levels for group Z. As a result of the failure of induced antibody to increase bactericidal activity, only 40% of vaccinees achieved a  $>2$  log<sub>2</sub> increase in lytic activity against group Z.

The independence of the binding antibody response to the two polysaccharides demonstrates that humans respond independently to both epitopes within 29E polysaccharide. The induction of group Z polysaccharide antibody that depresses group Z bactericidal activity suggests that blocking antibody is directed at the cross-immunogenic epitope. The parallelism of the group Z and 29E responses after two weeks suggests that group 29E lytic antibody is specific for the unique epitope and has a short half-life.

A radioimmunoprecipitin (RIP) modification of the standard RABA was developed to quantify Ig isotypes in the vaccinees sera. Optimal conditions had not been established by the end of the contract period. Although quantification of the Ig isotype specific response could not be undertaken, the conclusion that the cross-immunogenic epitope in group 29E polysaccharide induces both IgA and IgG in ratios that vary by individual, whereas the unique epitope induces only IgM, would explain the observed discordance between binding and bactericidal activity and is feasible.

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<sup>5</sup>Griffiss, J.McL., Brandt, B.L., Altieri, P.L., Pier G.B., Berman S.L. Safety and immunogenicity of a group 29E meningococcal capsular polysaccharide vaccine in adults. *Infect. Immun.* 1983; 39:247-252.

An immunologic cross-reaction between the 29E capsular polysaccharide and enteric LPS was confirmed using two separate serologic assays.

1. Indirect hemagglutination

LPS was extracted from an Re isogenic mutant of S. minnesota strain SF 1111 by the chloroform-ether technique. After alkali-treatment, the LPS was used to sensitize sheep erythrocytes. Serum from a group 29E vaccinate was absorbed thrice with either the homologous S. minnesota Re strain or a group 29E N. meningitidis and tested before and after absorption by indirect hemagglutination. Both the S. minnesota Re and the N. meningitidis group 29E reduced the hemagglutinating titre from 1:16 to <1:2.

2. Indirect fluorescent antibody assay (IFA)

This same serum before and after absorption with the two strains was also tested in an IgM-specific indirect fluorescent antibody assay. A 3 log<sub>2</sub> reduction in titre of antibody was again effected by absorption with both bacteria.

The optimal conditions for IFA specific for IgM, IgA and IgG proved difficult to establish, since IgA binding to the immobilized bacteria blocked IgM binding, and IgG binding competed with both IgM and IgA binding. In essence, this is a problem of reproducibly establishing extreme antigen excess. Since it is impossible to accurately quantify the number of bacteria immobilized on a slide, the IFA approach was not pursued.

3. RABA:

Absorption of the serum did not diminish its binding of radiolabelled group 29E polysaccharide in the RABA. On the basis of the low titre of cross-reactive antibody in the vaccinate's serum as compared with the titre of antibody to the unique, KDO-dependent epitope in group 29E polysaccharide in that serum, and the inability of the group Z cross-reactive antibody to distinguish between hexosamines, we conclude that the cross-reaction between the group 29E polysaccharide and enteric LPS involves glucosamine in the lipid A backbone rather than the carboxylic acid residue of KDO.

III. Conclusions.

Data that were developed during the fifteen months of this contract have demonstrated that: 1) the 8021 capsular polysaccharide is not a suitable vaccine candidate and that further development of group Y and W135 polysaccharide vaccines is not necessary. 2) that humans respond to two epitopes contained within the group 29E capsular polysaccharide and that the response to them varies by individual and in Ig isotype; 3) that neither the group 29E nor group Z capsular polysaccharides should be considered for inclusion in a polyvalent meningococcal vaccine

until their immunochemistry has been more completely understood and 4) that the cross-reaction observed between 29E capsular polysaccharide and enteric LPS is part of a larger set of cross-reactions, the precise molecular basis of which remains obscure.

END

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